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Assessing Readiness of Black Spruce Container Seedlings for Frozen Storage using Electrical Impedance, Oscilloscope Square Wave and Frost Hardiness Techniques

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Introduction

Frozen storage is an accepted practice for overwintering some species of bareroot nursery stock in Ontario, but container seedlings are almost without exception overwintered outside. Techniques for hardening container seedlings for removal from greenhouses have been developed (Colombo et al. 1984), but even hardened seedlings may be damaged during winters without adequate snowcover, especially if exposure in the holding area is not minimized. One means of avoiding these losses is controlled environment frozen overwinter storage of container seedlings. Overwinter frozen storage of container stock is also potentially important because it would allow crops to be kept growing (i.e. delay bud initiation) safely later in the year to reach minimum acceptable height.

Successful frozen storage requires proper preconditioning of seedlings (Hocking and Nyland 1971), and therefore some means of measuring readiness for storage is necessary. This note examines the use of three techniques for evaluating the readiness for storage of black spruce (Picea mariana (Mill) B.S.P.) container seedlings.

Materials and Methods

Black spruce seedlings were sampled from the operational crops grown in FH408 paperpots in greenhouses at the Swastika Tree Nursery in 1983. Seedlings were grown using standard procedures similar to those described by Carlson (1983). Seeds from a 32-3200 northeastern Ontario seedlot were sown on a peat-vermiculite medium between April 15 and 21, 1983. At the end of the growing period, three hardening regimes were used: 1/ **PR**. The former "production run" procedure, in which seedlings were moved outside July 18 and subjected to natural daylengths and ambient temperature, 2/ **EG**. Extended greenhouse culture (Colombo et al. 1984), i.e., natural daylengths and warm temperatures inside the greenhouse, and 3/ **SD**. Short daylengths (8 h) in the greenhouse from July 18 to August 16 to initiate bud development, followed by extended greenhouse culture at normal

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daylengths. Minimum greenhouse temperatures during extended greenhouse culture were 20°C.

Seedlings were collected at random weekly from August 11 to November 11, 1983 to determine stem electrical impedance, oscilloscope square wave pattern and shoot tip frost hardiness. Every week 25 seedlings from each hardening regime were placed in frozen storage at -1° to -3°C. The container plugs of seedlings for storage were extracted from the tray, and the seedlings were sealed in a polyethylene-lined kraft paper bag before being placed in storage.

Shoot tips of 20 seedlings per hardening regime were collected in early November following the completion of bud development and were dissected and needle primordia counted using procedures of Colombo and Odlum (1984).

Stem electrical impedance was measured on 20 seedlings per week, except in the first week when only 10 seedlings were used. Impedance was measured on the stem just above the cotyledons, using a 1 kHz impedance bridge (Glerum 1980). Two stainless steel electrodes, spaced 1 cm apart, were passed through the approximate centre of the stem. Impedance values were adjusted to a value which would correspond to a stem diameter of 2.0 mm, using the linear correlation between impedance and the reciprocal of diameter squared (Racey 1984).

Stem square wave patterns were determined using procedures similar to those of Day *et al.* (1985). Four stainless steel electrodes, aligned and spaced 1 cm apart, were passed through the approximate centre of the stem just above the cotyledons. The effect of the stem tissues on the square wave was visualized using a 15 kHz oscilloscope. Square wave patterns of 20 seedlings per week (except for 10 seedlings in week 1) were analyzed as having a left hand peak, a right hand peak, or a wave form in transition between left and right hand peaks (Racey 1984, Day *et al.* 1985).

Frost hardiness was determined on 2-3 cm of the shoot tip using the electrical conductivity-diffusate method as described in detail by Colombo *et al.* (1984). Detached shoot tips were frozen at a rate of 5°C per hour to a minimum temperature of -15°C. Two replicate groups of fifteen main-stem shoot tips of seedlings from each hardening regime were frozen. A third group of non-frozen samples of 15 shoot tips served as a control. Frozen and control samples were incubated in distilled water for 24 hours prior to measuring electrical conductivity of the incubating solution. Index of injury (I_t) was calculated as:

$$I_t = \frac{RC_f - RC_c}{1 - \frac{RC_c}{100}} \qquad [1]$$

where RC = electrical conductivity of samples relative to total electrical conductivity (after heat-killing), expressed on a percentage basis.

RC_f = relative conductivity of frozen samples.
and RC_c = relative conductivity of control samples.

Visible symptoms of damage to the stored seedlings were evaluated on November 28 and 29. Seedlings thawed 12 hours at room temperature sealed in their bags prior to damage assessment. Seedlings were classed as damaged if the terminal of the shoot was flaccid and dehydrated and the inner bark was discoloured.

Results and Discussion

In this trial, neither of the electrical techniques of assessing readiness for storage adequately detected readiness for storage. Square wave patterns bore no relation to readiness for storage, as all seedlings measured on all dates and from all regimes showed left-hand wave trace peaks, which apparently indicates "active" seedlings (Day et al. 1985). Several other studies have also found that square wave patterns bear no relation to dormancy or frost hardiness (Askren and Hermann 1979, Holbo et al. 1981, Rietveld et al. 1982, Jaramillo 1984). To our knowledge there are no published studies in which readiness for storage has been predicted by the oscilloscope square wave technique.

Although the stem impedance technique has been shown to be a good indicator of the readiness of bareroot stock for frozen overwinter storage (Racey 1984), for container stock used in this trial there was no one threshold impedance value common to all treatments which indicated the ability of the seedlings to be stored without damage (Fig. 1). Instead, for EG seedlings, storage without damage was possible beginning November 4, when impedance was 324 kΩ , whereas for the PR and SD treatments safe storage was possible when impedance was 157 kΩ and 146 kΩ respectively. The basis of the "safe-lifting zones" of Racey (1984) is that each species in each nursery has a characteristic minimum impedance value associated with readiness for storage. Further testing of the impedance technique with container stock is warranted.

In comparison, the index of injury technique for assessing frost hardiness reliably predicted readiness for storage. Seedlings of all hardening regimes which were put in frozen storage after having become hardy to -15°C (as indicated by two consecutive weeks with an index of injury of 5% or less (Colombo et al. 1984)) suffered no damage (Fig. 2). In British Columbia, where frozen storage of container stock is a routine practice, readiness for storage is reliably predicted using freezing tests to -18°C (Simpson 1986). Our results suggest that a similar criterion may reliably predict readiness of black spruce container stock for storage.

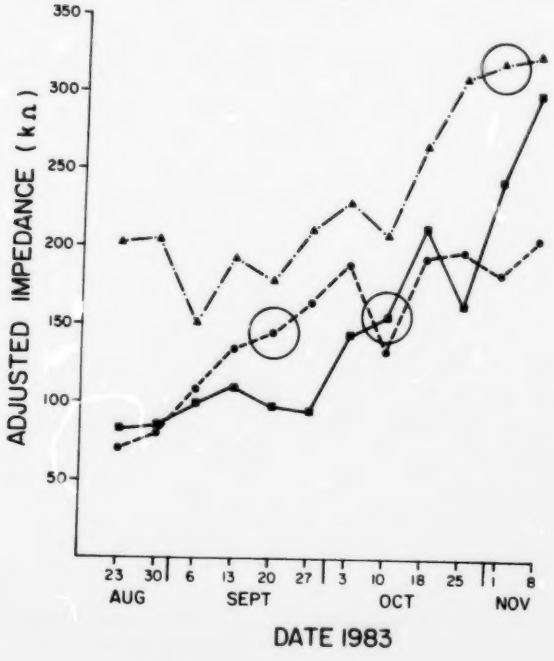

Figure 1 Adjusted electrical impedance of black spruce container seedlings during hardening. PR, ■ ; SD, ● ; EG, ▲ . Circled points indicate dates on which storage without damage began. Details of hardening regimes are given in text.

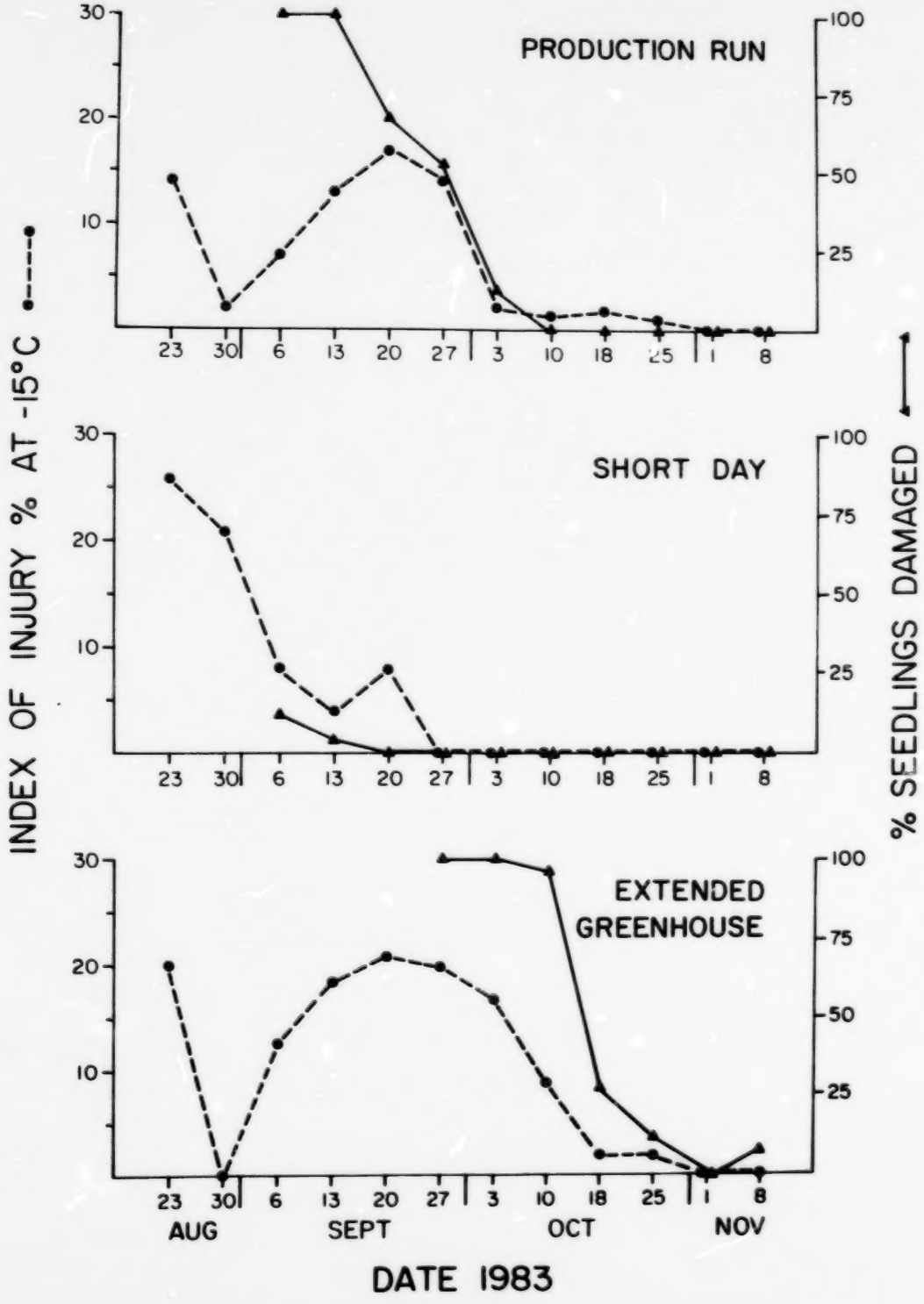

Figure 2 Index of injury and percentage of seedlings damaged during frozen storage of black spruce container stock of three hardening regimes.

The results for electric impedance and frost hardiness are promising but must be considered as preliminary. The preferred method for assessing damage and mortality following frozen storage is through pot and field planting studies. In this first trial, such assessments were not part of the original project plan and stock was therefore not available for planting.

Hardening regime significantly affected the date of the development of frost hardiness. Cool temperatures and natural daylengths (PR hardening regime) resulted in earlier development of frost hardiness than warm temperatures and natural daylengths (EG seedlings). However, bud development of PR seedlings was only 178 needle primordia compared to 260 primordia for EG seedlings. Short daylengths were most effective in speeding frost hardiness development and readiness for storage, as SD seedlings (with 200 needle primordia) were ready for storage by October 3 compared to October 25 for extended greenhouse culture stock.

Frozen storage of container stock would add flexibility to the double-crop system of producing containerized tree seedlings. Normally, the spring-crop is sown in February and grown for about 15 weeks until the end of May. The second (summer) crop is June-sown and grown approximately 12 weeks until the end of August. Shortcomings in growing regimes or weather conditions reduce the likelihood of seedlings reaching acceptable heights within these production periods. Previously in such cases the summer crop has been kept growing in height beyond the end of August. However, crops kept growing beyond August suffer an increased risk of overwinter damage due to the likelihood of very cold day and night temperatures, which, when accompanied by high winds, are liable to result in freezing damage, as the seedlings will not be ready to be moved outside until at earliest the end of November. In addition, while it is possible to harden seedling shoots to withstand low temperatures inside a greenhouse using extended greenhouse culture (Colombo et al. 1984), root freezing damage may occur under some conditions. Our knowledge of the development of root hardiness is incomplete.

If facilities for frozen storage of container stock and suitable measures to determine readiness for storage were available, seedlings could be kept growing in the greenhouse as long as necessary and then moved directly into storage. This would also benefit planting programs, since seedlings could be kept inactive in storage until needed in the spring. As it now stands, seedlings overwintered outside have often begun shoot elongation by the time of planting.

In conclusion, the frost hardening of the shoots of containerized black spruce seedlings to -15°C appears to be a consistent prerequisite for frozen storage. Extended greenhouse culture of spruce container stock can thus be tentatively used in conjunction with freezing tests to determine readiness for frozen storage. In this way container nurseries could reliably produce two crops of spruce seedlings of acceptable size and optimum morphology per year, without the risks of winter damage to the second-crop which accompanies the current practice of out-of-doors storage.

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